

# Bronchial Challenge With Tri a 14 as an Alternative Diagnostic Test for Baker's Asthma

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## ■ Abstract

**Background:** Baker's asthma (BA) is the most prevalent occupational respiratory disease in developed countries. It is caused by inhalation of wheat dust in the working environment and affects 1%-10% of workers in the baking industry. Diagnosis of BA is based on bronchial challenge with wheat, a technique that carries a high risk for patients. The wheat lipid transfer protein Tri a 14 is a major allergen in BA. **Objective:** The aim of our study was to characterize Tri a 14 as a marker of BA in order to prevent patients from having to undergo bronchial challenge with wheat.

**Methods:** The study population comprised 55 patients selected at the Rio Hortega Hospital, Valladolid, Spain. Patients with BA were diagnosed using a skin prick test (SPT) with wheat and Tri a 14 and bronchial challenge test (BCT) with wheat. Patients with food allergy had a clear clinical history of allergy to peach confirmed by positive SPT to peach extract and Pru p 3.

**Results:** All patients in the BA group had a positive SPT result with wheat (100%), and most had positive results with Tri a 14 (95%). A positive BCT result with Tri a 14 was also observed in 22 of 27 of the patients with BA (82%). The response to Tri a 14 was specifically associated with BA.

**Conclusion:** Tri a 14 is a good marker of BA and can be used in SPT and BCT as an alternative diagnostic method, thus avoiding bronchial challenge with wheat and reducing the risk associated with this technique.

**Key words:** Baker's asthma. Lipid transfer protein. Diagnostic marker. Tri a 14.

## ■ Resumen

**Antecedentes:** El asma del panadero (BA) es la enfermedad respiratoria ocupacional más frecuente en los países occidentales. Está causada por la inhalación diaria de harina de trigo en el entorno de trabajo, afectando entre 1-10% de los trabajadores de la industria panadera. El diagnóstico de BA se basa en la provocación bronquial con trigo, una técnica de alto riesgo para los pacientes. La proteína de transferencia de lípidos de trigo (LTP) Tri a 14 ha sido descrita como alérgeno principal en esta patología.

**Objetivo:** El objetivo de nuestro estudio ha sido caracterizar Tri a 14 como marcador de BA, y así evitar la provocación bronquial con trigo en estos pacientes.

**Métodos:** Para ello, se seleccionaron cincuenta y cinco pacientes en el Hospital Río Hortega de Valladolid, España.

**Resultados:** Los pacientes diagnosticados con BA mostraron prueba cutánea (SPT) positiva a trigo (100%) y la mayoría también a Tri a 14 (95%). Todos ellos, fueron sometidos a provocación bronquial con Tri a 14, observándose un resultado positivo en 22/27 de los sujetos evaluados (82%).

**Conclusiones:** En base a esto, se puede concluir que Tri a 14 es un buen marcador de BA, y podría ser utilizado en SPT y provocación bronquial como método diagnóstico, reduciendo el uso de la provocación bronquial con trigo y el riesgo asociado a esta técnica.

**Palabras clave:** Asma del panadero. Proteína de transferencia de lípidos. Alérgeno marcador. Tri a 14. Diagnóstico de alergia. LTP.

## Introduction

Occupational asthma causes more than 10% of all cases of asthma in adults, and baker's asthma (BA) is the most prevalent occupational respiratory disease in Western countries [1]. BA is a complex disease induced by inhalation of wheat dust in the working environment. It affects approximately 1%-10% of workers in the baking industry [2]. Many workers in the milling, baking, and food processing industries are exposed to wheat dust in the atmosphere and are therefore at high risk of developing respiratory allergy. However, this allergy is often misdiagnosed, with significant legal, economic, and health consequences for affected patients [3].

Current methods for the diagnosis of BA are limited. Bronchial challenge test (BCT) is considered the gold standard, although it can only be performed in centers with specialized equipment and trained personnel and is potentially dangerous for the patient [1]. In vitro testing (specific IgE assays) has poor predictability and specificity, possibly because of insufficient purity of the wheat extracts used or the absence of all major allergens in the extracts [3,4]. The basophil activation test has been proposed as a reliable in vitro technique for diagnosis of immediate food allergy (FA) to wheat in children [4], although it is not widely used for diagnosis of BA.

In recent years, some authors have aimed to design a panel of wheat allergens for diagnosis of patients with BA that would obviate specific bronchial challenge [5,6]. Over 30 wheat allergens have been associated with BA, and many have also been implicated in food allergy [7]. The introduction of microarray techniques featuring a large panel of purified allergens has proven a major advance in the diagnosis of allergic diseases [8]. We previously applied this approach in the diagnosis of patients with BA [9] and found that Tri a 14 was recognized only by patients with BA, but not by those diagnosed with wheat allergy.

The wheat lipid transfer protein (LTP) Tri a 14 is a major allergen in BA [10,11]. Tri a 14 belongs to a plant LTP family that has been extensively shown to include food allergens. In the case of BA, 45% of Spanish patients had a positive skin prick test (SPT) result to Pru p 3, although most could consume peach without experiencing symptoms [12]. These results point to primary sensitization through wheat dust inhalation, followed by development of allergy after oral intake due to cross-reactivity between Pru p 3 and Tri a 14. An analysis of the molecular basis of the cross-reactivity between peach and wheat revealed an overlapping IgE-binding region in both proteins [13].

The aim of our study was to characterize Tri a 14 as a marker of BA in order to prevent patients from having to undergo bronchial challenge with wheat. The response to Tri a 14 was compared with that of patients with FA.

## Methods

### Study Population

Two groups of patients were selected at Rio Hortega Hospital in Valladolid, Spain. Patients in the BA group (patients 1 to 37, Table) had to have a convincing clinical history of wheat-induced respiratory allergy, a positive response in the

SPT, and positive result in a BCT with wheat. Patients in the FA group (patients 1 to 18, Table) had to have a clinical history of peach allergy and positive SPT result with peach extract (ALK-Abelló). These patients also had to have 1 of the following: oral allergy syndrome, urticaria-angioedema, gastrointestinal symptoms (diarrhea, vomiting), and respiratory allergy (rhinitis-asthma) after ingestion of peach.

Five nonallergic individuals were included as a control group for the BCT. No response was observed in this group. The Ethic Committees of the Technical University of Madrid and Rio Hortega Hospital in Valladolid approved the study. All patients and controls gave their written informed consent to participate.

### Skin Prick Testing

SPT was performed according to standard procedures following the recommendations of the European Academy of Allergy and Clinical Immunology [14]. All patients underwent SPT using an in-house wheat extract (10 mM PBS, 0.5M NaCl, pH 7.4), a commercial peach extract (ALK-Abelló), and purified proteins (native and recombinant forms of Tri a 14 and Pru p 3). SPT was performed by testing the wheat and peach extracts up to 1 mg/mL and the purified proteins up to 20 µg/mL [15]. One sterile device was used for each test. Histamine phosphate (10 mg/mL) and sterile 0.9% saline were used as positive and negative controls, respectively. A mean diameter  $\geq 3$  mm greater than the negative control 15 minutes after puncture was considered a positive result. The panel of common aeroallergens (ALK-Abelló) included grass pollen, olive tree pollen, dust mites, molds, and foods (nuts, barley, and fruits [kiwi, apple, peach]), and spices.

### Bronchial Challenge Testing

BCT was performed double blind as described by Palacin et al [10], with modifications [16,17]. An in-house wheat extract with a protein concentration of 5 mg/mL and fresh peach juice were used. Spirometry was performed following the recommendations of the American Thoracic Society/European Respiratory Society [18]. Aerosolized particles generated using a continuous pressurized DeVilbiss 646 nebulizer were inhaled for 2 minutes at a normal breathing volume, starting with control PBS and followed—at 10-minute intervals—by increasing concentrations of the extract. The dose was determined by end-point titration based on the first dilution with a positive SPT response. A positive response was defined as  $>20\%$  fall from baseline in forced expiratory volume in the first second (FEV<sub>1</sub>). After specific BCT, hourly peak expiratory flow measurements were recorded for 9 hours. BCT was performed with the same extract in 5 healthy controls, all of whom had negative results.

To confirm peach allergy, oral challenge was performed with whole peach juice (skin plus pulp) freshly liquefied in a blender. The challenge began with a 1:100 dilution of the aliquot of juice that had generated a wheal measuring at least  $3 \times 3$  mm<sup>2</sup>.

### Wheat Extract

Whole seeds of bread wheat (*Triticum aestivum*, Astral cultivar) were ground, defatted with cold acetone (1:5 [wt/

Table. Demographic and Clinical Data of Study Patients

Patient	No.Age/Sex	Symptoms	SPT (mm <sup>2</sup> ) <sup>a</sup>				Oral Challenge <sup>b</sup>		Bronchial Challenge		
			Wheat	Tri a 14	Peach	Pru p 3	Wheat	Peach	Wheat	Peach	Tri a 14
BA1	37/M	As	20	28	0	0	0	0	1	0	1
BA2	18/M	As	78	0	78	68	1	ND	1	1	1
BA3	46/F	As, An	82	84	42	48	1	ND	1	0	ND
BA4	31/F	As, An	28	32	28	28	0	ND	1	0	ND
BA5	20/M	As	38	28	0	0	0	0	1	0	1
BA6	46/M	As	29	30	0	0	0	0	1	0	1
BA7	32/F	As, U, OAS	36	26	0	0	0	0	1	0	1
BA8	56/M	As	20	22	0	0	0	0	1	0	0
BA9	25/M	As, U	20	24	0	0	0	0	1	0	1
BA10	18/M	As, An	38	32	32	0	1	0	1	0	ND
BA11	25/F	As	28	26	0	0	0	0	1	0	1
BA12	22/M	As, An	23	24	0	0	1	0	1	0	ND
BA13	41/M	As, An	32	28	22	24	1	1	1	0	ND
BA14	34/M	As	20	41	0	0	0	0	1	0	1
BA15	19/M	An	24	22	62	60	1	1	1	1	ND
BA16	28/M	As	42	32	34	28	1	1	1	1	1
BA17	41/M	An	38	28	32	28	1	ND	1	1	ND
BA18	19/F	An	24	24	62	60	1	ND	1	1	ND
BA19	35/F	An	36	32	42	46	1	1	1	1	ND
BA20	27/M	As	24	24	0	0	0	0	1	0	1
BA21	19/M	As	32	32	0	0	0	0	1	0	1
BA22	40/M	As	26	26	0	0	0	0	1	0	0
BA23	27/M	Rh, As	22	26	0	0	0	0	1	0	0
BA24	36/M	Rh, As	20	22	0	0	0	1	1	0	1
BA25	37/M	As, U	42	42	0	20	0	0	1	0	1
BA26	38/M	As	22	22	0	0	0	0	1	0	1
BA27	41/M	As	24	0	0	0	0	0	1	0	1
BA28	65/M	Rh, As	22	22	0	0	0	0	1	0	0
BA29	40/M	Rh, As	22	22	0	0	0	0	1	0	1
BA30	52/M	As	32	32	0	0	0	0	1	0	1
BA31	34/M	Rh, As	52	52	0	0	0	0	1	0	1
BA32	26/M	As	34	34	0	0	0	0	1	0	0
BA33	41/M	As	22	22	0	0	0	0	1	0	1
BA34	20/M	As	32	32	0	0	0	0	1	0	ND
BA35	22/M	As	26	26	0	0	0	0	1	0	1
BA36	41/M	As	26	28	0	0	0	0	1	0	1
BA37	38/M	As	28	28	0	0	0	0	1	0	1
FA1	22/F	As	28	30	22	0	1	0	0	0	0
FA2	22/F	As, An	42	46	48	29	1	1	0	0	ND
FA3	18/M	An	0	42	38	32	0	ND	0	0	ND
FA4	18/F	An	0	40	22	24	0	1	0	0	ND
FA5	25/F	An	0	40	49	38	0	1	0	0	ND
FA6	21/F	An	74	63	32	39	1	1	0	0	ND
FA7	20/M	As, An	0	40	52	48	0	1	0	1	ND
FA8	20/F	An	21	43	86	62	0	1	0	ND	ND
FA9	24/M	As	22	0	42	40	0	0	0	0	0
FA10	47/F	An	0	0	43	46	0	1	0	0	0
FA11	22/M	Rh, An	0	0	22	29	1	1	0	0	0
FA12	21/M	A, U, OAS	22	0	20	22	1	ND	0	0	ND
FA13	20/M	An	40	23	42	42	0	1	0	0	0
FA14	18/M	U, OAS	0	0	22	22	0	1	0	0	0
FA15	18/M	OAS	0	0	34	34	0	1	0	0	0
FA16	20/M	OAS	0	0	22	22	0	1	0	0	0
FA17	23/F	U, OAS	0	0	32	32	0	1	0	0	0
FA18	22/M	U, OAS	0	0	24	25	0	1	0	0	ND

Abbreviations: An, anaphylaxis; As, asthma; BA, baker's asthma; FA, food allergy; ND, not determined; OAS, oral allergy syndrome; Rh, rhinitis; SPT, skin prick test; U, urticaria.

<sup>a</sup>Area  $\geq 20$  mm<sup>2</sup> was considered positive.

<sup>b</sup>1=positive; 0=negative.

vol] for 1 hour at 4°C), and, after drying, extracted with 0.1 mol/L Tris-HCl (pH 7.5) and 10 mmol/L EDTA (1:5 [wt/vol] for 1 hour at 4°C). After centrifugation (9000g for 30 minutes at 4°C), the supernatant was dialyzed against H<sub>2</sub>O (cutoff, 3.5 kDa) and freeze-dried. This crude extract was used for the SPTs. The protein concentration was quantified according to the Bradford method [19].

### Isolation and Characterization of Tri a 14

The wheat bran extract was fractionated by means of cation exchange chromatography on a Sep-Pak Accell Plus CM cartridge (Waters) using 10 mmol/L ammonium acetate (pH 6.8) as the equilibration buffer. The retained material, which was enriched in Tri a 14, was then eluted with ammonium acetate (50 mmol/L, pH 6.8), dialyzed, and freeze-dried. This Tri a 14-enriched fraction was further separated by reverse-phase high-performance liquid chromatography on a Nucleosil 300-C4 column (Scharlau Science; 8×250 mm; particle size, 5 mm) and elution with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (10% to 100% in 150 minutes; 1 mL/min). Chromatographic fractions containing Tri a 14 were located by immunodetection after SDS-PAGE with both anti-peach LTP antibody and a serum pool from patients with BA. These fractions were then pooled and freeze-dried. Pru p 3 was purified as previously described [20]. The allergens isolated were quantified using a commercial bicinchoninic acid test (ThermoFisher Scientific). SDS-PAGE was performed on Bio-Rad Miniprotein III System gels (15% polyacrylamide), according to the method of Laemmli. Bands corresponding to Tri a 14 in bran and flour extracts were quantified using the GelDoc 2000 System and Quantity One software (both from BioRad). N-terminal amino acid sequencing was carried out using standard methods with an Applied Biosystems 477A gas-phase sequencer.

### Statistical Analysis

Differences in the frequencies of positive response were analyzed using contingency tables and the  $\chi^2$  test. Quantitative variables (response to BCT vs SPT result in mm<sup>2</sup>) were compared using the Pearson correlation. The predictive value

of Tri a 14 in SPT and BCT was calculated using receiver operating characteristic (ROC) curves and analysis of the area under the curve (AUC).  $P < .05$  was considered significant.

## Results

In addition to a positive BCT result with wheat, all BA patients in our study had a positive SPT result with wheat (100%) and most also had a positive result with Tri a 14 (95%). We observed an association between BA and a positive response to Tri a 14 by SPT ( $\chi^2$  test, 0.52;  $P < .0001$ ).

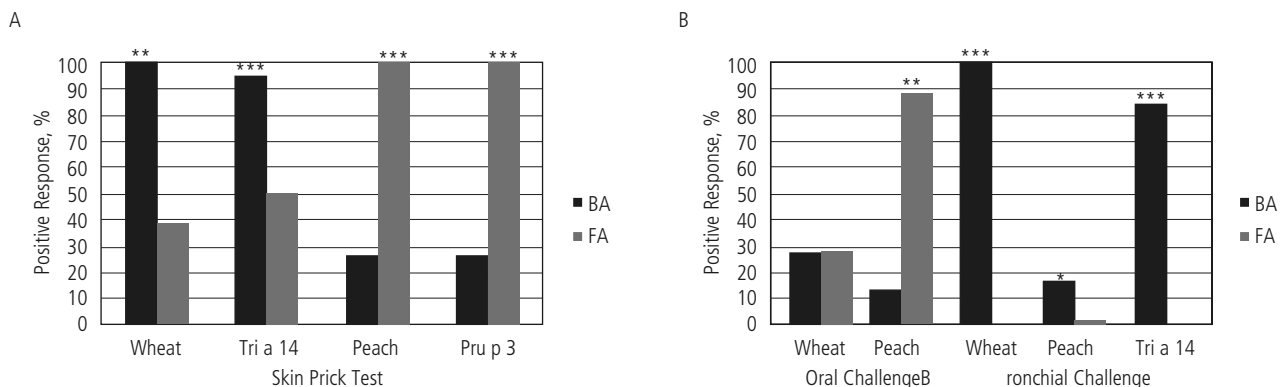
Similarly, a positive BCT result with Tri a 14 was observed in 22 of the 27 patients with a positive response to the BCT (82%). The results of both tests (SPT and BCT) were compared for each patient, and a strong correlation was observed (Pearson, 0.83;  $P < .0001$ ). By contrast, most patients in this group had a negative response to peach in the oral challenge (only 5 positive results in 32 patients) to peach juice in the BCT (only 6 positive results in 37 patients) (Figure, A and B).

In contrast with the results for the BA patients, all patients in the FA group reacted to peach and Pru p 3 by SPT, although 50% also had a positive response to Tri a 14 by SPT. Moreover, 87.5% (14/16) of the patients who underwent oral challenge with peach experienced symptoms after the test. In the BCT, only 1 FA patient (6%) showed a positive result with peach but did not react to wheat or Tri a 14 (Figure, A and B).

Receiver operating characteristic (ROC) curves were constructed to compare the SPT and BCT response to Tri a 14 with the response to wheat to determine the validity of Tri a 14 as a diagnostic tool in BA. The area under the curve (AUC) was 0.5201 for SPT and 0.5926 for BCT. The values obtained are within the reference range. Thus, Tri a 14 seemed to be a specific marker of BA in the BCT.

## Discussion

Occupational allergic respiratory disorders such as BA are often misdiagnosed, with significant legal, economic, and health consequences for affected patients [1,3]. BA is the leading cause of occupational respiratory disease in developed



**Figure.** Positive response of baker's asthma (BA) and food allergy (FA) patients when tested with wheat, peach, Tri a 14, and Pru p 3 by skin prick test (A) and by oral and bronchial challenge testing (B). Statistical differences are shown above the bars. The  $\chi^2$  test was used for all comparisons. P value: \* $< .05$ ; \*\* $< .001$ ; \*\*\* $< .0001$ .

countries [21]. It is caused by the inhalation of wheat flour allergens, which does not seem to be influenced by local environmental allergens (pollen), and appears to be associated with a specific allergen sensitization profile that is different from that observed in patients with wheat-induced food allergy.

Our study was performed in central Spain, a region with large areas of grass crops. Tri a 14 was recognized by most BA patients (95% by SPT and 82% by BCT). In fact, Tri a 14 has been specifically associated with respiratory allergy to wheat but not with FA to wheat [8]. In the case of FA, a high percentage (50%) of patients also showed a positive reaction to Tri a 14 in the SPT, possibly owing to cross-reactivity between Tri a 14 and Pru p 3, as described previously in FA [12]. By contrast, this high reactivity was not confirmed by oral and bronchial challenge testing.

We previously found that >60% of Spanish BA patients from different regions reacted to Tri a 14 both in vitro and in vivo, regardless of local pollen distribution [9,12]. Similarly, in the Czech Republic, Sotkovsky et al [11] found that 64% of BA patients presented IgE binding to Tri a 14. These results indicated that Tri a 14 is a major allergen involved in the development of BA, in contrast to wheat FA, where Tri a 14 seems to have a minor role [9]. Curiously, in a recently published study [23] on nonceliac wheat-allergic children, none of the patients presented a positive response to Tri a 14. Similarly, <10% of adult patients with allergy to plant foods had a positive response to Tri a 14 [8].

In recent years, some studies have aimed to design a panel of wheat allergens for the diagnosis of patients with BA in order to avoid specific bronchial challenge with wheat owing to its potential risk and technical requirements. Sander et al [22] produced a panel of 17 recombinant allergens associated with BA. However, the frequencies of sensitization observed were low, and no major allergens were detected. Sotkovsky et al [11] studied the IgE binding of wheat allergens using natural forms. Gomez Casado et al [9] identified more than 20 allergens, but only Tri a 14, Tri a TLP, and tritin were recognized by more than 50% of sensitized patients (64%, 100%, and 100%, respectively). Consequently, these were considered major allergens. However, only Tri a 14 seemed to be BA-specific.

The specificity of Tri a 14 as a diagnostic marker of BA has been demonstrated both in vitro and in vivo [9]. In this study, Tri a 14 presented high sensitivity, as it was recognized by 95% and 82% of BA patients in SPT and BCT, respectively. The predictive value of Tri a 14 as a marker of wheat-induced respiratory allergy was demonstrated by BCT, as described elsewhere [10].

Tri a 14 proved to be a good tool for the diagnosis of BA. Therefore, BCT with wheat could be avoided, at least in those patients who react to Tri a 14, thus reducing the problems associated with the use of complete extracts in the diagnosis of this disorder.

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### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### References

- Salcedo G, Quirce S, Diaz-Perales A. Wheat allergens associated with Baker's asthma. *J Invest Allergol Clin Immunol*. 2011;21:81-92.
- Jacobs JH, Meijster T, Meijer E, Suarthana E, Heederik D. Wheat allergen exposure and the prevalence of work-related sensitization and allergy in bakery workers. *Allergy*. 2008;63:1597-604.
- van Kampen V, Merget R, Rabstein S, Sander I, Bruening T, Broding HC, Keller C, Muesken H, Overlack A, Schultze-Werninghaus G, Walusiak J, Raulf-Heimsoth M. Comparison of wheat and rye flour solutions for skin prick testing: a multicentre study (Stad 1). *Clin Exp Allergy*. 2009;39:1896-902.
- Tokuda R, Nagao M, Hiraguchi Y, Hosoki K, Matsuda T, Kouno K, Morita E, Fujisawa T. Antigen-induced expression of CD203c on basophils predicts IgE-mediated wheat allergy. *Allergol Int*. 2009;58:193-9.
- Pahr S, Constantin C, Mari A, Scheiblhofer S, Thalhamer J, Ebner C, Vrtala S, Mittermann I, Valenta R. Molecular characterization of wheat allergens specifically recognized by patients suffering from wheat-induced respiratory allergy. *Clin Exp Allergy*. 2012;42:597-609.
- Wiszniewska M, Nowakowska-Swirta E, Palczynski C, Walusiak-Skorupa J. Diagnosing of bakers' respiratory allergy: is specific inhalation challenge test essential? *Allergy Asthma Proc*. 2011;32:111-8.
- Tatham AS, Shewry PR. Allergens to wheat and related cereals. *Clin Exp Allergy*. 2008;38:1712-26.
- Palacín A, Gómez-Casado C, Rivas LA, Aguirre J, Tordesillas L, Bartra J, Blanco C, Carrillo T, Cuesta-Herranz J, de Frutos C, Alvarez-Eire GG, Fernández FJ, Gamboa P, Muñoz R, Sánchez-Monge R, Sirvent S, Torres MJ, Varela-Losada S, Rodríguez R, Parro V, Blanca M, Salcedo G, Díaz-Perales A. Graph based study of allergen cross-reactivity of plant lipid transfer proteins (LTPs) using microarray in a multicenter study. *PLoS One*. 2012;7:e50799.
- Gómez-Casado C, Garrido-Arandia M, Pereira C, Catarino M, Parro V, Armentia A, Quirce S, Armentia B, Barranco P, Blanca M, Campo P, Díaz-Perales A. Component-resolved diagnosis of wheat flour allergy in baker's asthma. *J Allergy Clin Immunol*. 2014;134:480-3.
- Palacín A1, Varela J, Quirce S, del Pozo V, Tordesillas L, Barranco P, Fernandez-Nieto M, Sastre J, Diaz-Perales A, Salcedo G. Recombinant lipid transfer protein Tri a 14: a novel heat and proteolytic resistant tool for the diagnosis of baker's asthma. *Clin Exp Allergy*. 2009;39:1267-76.
- Sotkovský P1, Sklenář J, Halada P, Cinová J, Setinová I, Kainarová A, Goliáš J, Pavlásková K, Honzová S, Tučková L. A new approach to the isolation and characterization of wheat flour allergens. *Clin Exp Allergy*. 2011;41:1031-43.
- Palacín A1, Quirce S, Armentia A, Fernández-Nieto M, Pacios LF, Asensio T, Sastre J, Diaz-Perales A, Salcedo G. Wheat lipid transfer protein is a major allergen associated with baker's asthma. *J Allergy Clin Immunol*. 2007;120(5):1132-8.



13. Tordesillas L1, Pacios LF, Palacin A, Quirce S, Armentia A, Barber D, Salcedo G, Diaz-Perales A. Molecular basis of allergen cross-reactivity: non-specific lipid transfer proteins from wheat flour and peach fruit as models. *Mol Immunol*. 2009;47(2-3):534-40.
14. Position Paper: Allergen Standardization and skin tests. The European Academy of Allergy and Clinical Immunology. *Allergy*. 1993;48:49-54.
15. van Kampen V1, de Blay F, Folletti I, Kobierski P, Moscato G, Olivieri M, Quirce S, Sastre J, Walusiak-Skorupa J, Raulf-Heimsoth M. EAACI position paper: skin prick testing in the diagnosis of occupational type I allergies. *Allergy*. 2013;68:580-4.
16. Armentia A, Sanchez-Monge R, Gomez L, Barber D, Salcedo G. In vivo allergenic activities of eleven purified members of a major allergen family from wheat and barley flour. *Clin Exp Allergy*. 1993;23:410-5.
17. Panzani R1, Armentia A, Lobo R, Postigo I, Martínez J, Arranz ML, Martín-Gil JF, Fernández JC. Tolerance mechanisms in response to antigens responsible for baker's asthma in different exposed people. *J Asthma*. 2008;45:333-8.
18. Miller MR1, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. Standardisation of spirometry. *Eur Resp J*. 2005;26(2):319-38.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248-54.
20. Diaz-Perales A, Garcia-Casado G, Sanchez-Monge R, Garcia-Selles FJ, Barber D, Salcedo G. cDNA cloning and heterologous expression of the major allergens from peach and apple belonging to the lipid-transfer protein family. *Clin Exp Allergy*. 2002;32:87-92.
21. Brant A. Baker's asthma. *Curr Opin Allergy Clin Immunol*. 2007;7:152-5.
22. Sander I1, Rozynek P, Rihs HP, van Kampen V, Chew FT, Lee WS, Kotschy-Lang N, Merget R, Brüning T, Raulf-Heimsoth M. Multiple wheat flour allergens and cross-reactive carbohydrate determinants bind IgE in baker's asthma. *Allergy*. 66:1208-15.
23. Rodríguez del Río P, Díaz-Perales A, Sanchez-García S, Escudero C, do Santos P, Catarino M, Ibañez MD. Oral immunotherapy in children with IgE-mediated wheat allergy: outcome and molecular changes. *J Investig Allergol Clin Immunol*. 2014;24:240-8.

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